Activation of T cells by carbamazepine and carbamazepine metabolites

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Background: T-cell–mediated hypersensitivity is a rare but serious manifestation of drug therapy.
Objectives: To explore the mechanisms of drug presentation to T cells and the possibility that generation of metabolite-specific T cells may provoke cross-sensitization between drugs.
Methods: A lymphocyte transformation test was performed on 13 hypersensitive patients with carbamazepine, oxcarbazepine, and carbamazepine metabolites. Serial dilution experiments were performed to generate drug (metabolite)–specific T-cell clones to explore the structural basis of the T-cell response and mechanisms of antigen presentation. 3-Dimensional energy-minimized structures were generated by using computer modeling. The role of drug metabolism was analyzed with 1-aminobenzotriazole.
Results: Lymphocytes and T-cell clones proliferated with carbamazepine, oxcarbazepine, and some (carbamazepine 10,11 epoxide, 10-hydroxy carbamazepine) but not all stable carbamazepine metabolites. Structure activity studies using 29 carbamazepine (metabolite)–specific T-cell clones revealed 4 patterns of drug recognition, which could be explained by generation of preferred 3-dimensional structural conformations. T cells were stimulated by carbamazepine (metabolites) bound directly to MHC in the absence of processing. The activation threshold for T-cell proliferation varied between 5 minutes and 4 hours. 1-Aminobenzotriazole, which inhibits cytochrome P450 activity, did not prevent carbamazepine-related T-cell proliferation. Substitution of the terminal amine residue of carbamazepine with a methyl group diminished T-cell proliferation.
Conclusion: These data show that carbamazepine and certain stable carbamazepine metabolites stimulate T cells rapidly via a direct interaction with MHC and specific T-cell receptors.
Clinical implications: Some patients with a history of carbamazepine hypersensitivity possess T cells that cross-react with oxcarbazepine, providing a rationale for cross-sensitivity between the 2 drugs. (J Allergy Clin Immunol 2006;118:233-41.)

Key words: *Drug hypersensitivity, antigen presentation, T cells, cross-reactivity*

Administration of the anticonvulsant carbamazepine is associated with a high incidence of hypersensitivity reactions. These reactions are characterized clinically by delayed onset, the development of cutaneous manifestations, fever, internal organ involvement, and eosinophilia. Clinical cross-reactivity between carbamazepine and other anticonvulsants has been reported; however, the exact incidence and mechanistic basis are not known.

Blood lymphocytes from hypersensitive patients proliferate *in vitro* in the presence of carbamazepine and express skin homing receptors such as cutaneous lymphocyte antigen and CCR10.[1,2] T-cell cloning studies in carbamazepine hypersensitive patients have shown that carbamazepine is presented on HLA-DR and HLA-DQ molecules. Drug-specific T cells are mostly $CD4^+$, whereas drug stimulation results in the secretion of IFN-γ and perforin.[3]

The mechanisms by which drugs stimulate T cells are not fully understood. Moreover, such studies are confounded by the lack of drug metabolites and a lack of knowledge of the nature of the drug antigen. Traditionally, covalent binding of a drug or drug metabolite to autologous protein, followed by antigen processing and translocation of the derived peptides to the cell surface in association with MHC molecules, has been thought to be a prerequisite for T-cell receptor activation.[4,5] However, the vast majority of T cells that can be isolated from the peripheral blood of hypersensitive patients can be stimulated by the drug bound directly to the MHC in the absence of processing.[6-8] Furthermore, peptide elution experiments have revealed that drugs can interact directly with MHC and not an embedded peptide.[9] Thus, drug antigen specific T-cell receptors seem to be stimulated by chemicals approximately one third the size of a traditional MHC-associated peptide (ie, 200-350 d).

In the current investigations, we have explored the nature of the T-cell response by using T-cell clones from hypersensitive patients that respond to carbamazepine and/or stable carbamazepine metabolites. Further experiments were designed to explore the possibility that generation of metabolite-specific T cells may provoke cross-sensitization between drugs.

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Abbreviations used
APC: Antigen-presenting cell
SI: Stimulation index

METHODS

Donor characteristics

Blood was obtained and lymphocytes isolated from 12 patients hypersensitive to carbamazepine and 1 patient hypersensitive to oxcarbazepine. Approval for the study was obtained from Liverpool local research ethics committee, and informed consent was obtained. The clinical details of the patients are shown in Table I.

Chemicals and cell lines

EBV-transformed B-cell lines (referred to as antigen-presenting cells [APCs]) were generated by using supernatant from the EBV-producing cell line B9-58. All chemicals were prepared as stock solutions (10 mg/mL) in culture media and dimethyl sulfoxide (4:1 vol/vol). N-acetyl iminostilbene was synthesized by incubating iminostilbene (2.0020 g, 10.360 mmol) with acetic anhydride (15 mL) at 100°C for 5 hours. The crude product was extracted into ethyl acetate. After recrystallization from ethyl acetate/hexane, N-acetyl iminostilbene was obtained as a white solid (yield, 1.98 g, 81%; accurate mass, calculated: 236.10753, found: 236.10760; melting point: 110-112°C).

Determination of lymphocyte proliferation

Proliferation of patients' lymphocytes with carbamazepine, carbamazepine 10, 11 epoxide, 2-hydroxy carbamazepine, 3-hydroxy carbamazepine, 10-hydroxy carbamazepine, carbamazepine 10,11 *cis*-diol, carbamazepine 10,11 *trans*-diol, N-acetyl iminostilbene, and oxcarbazepine (all 10-100 μg/mL) was measured by using the lymphocyte transformation test, as described previously.[10] Proliferative responses were calculated as stimulation indices (SIs; cpm in drug-treated cultures/cpm in cultures with dimethyl sulfoxide alone).

Generation of T-cell clones

Carbamazepine (metabolite) stimulated lymphocytes were cloned by serial dilution using previously described methodology.[11] To test the specificity of the clones, T cells (0.5×10^5) were incubated with irradiated (60 Gy) APCs (0.1×10^5) and the drug (metabolite) that the lymphocytes were initially stimulated with. After 48 hours, [^{3}H] thymidine was added, and proliferation was measured 16 hours later by scintillation counting. CD phenotype and monoclonality was determined by flow cytometry.

Specificity of the T-cell clones

Irradiated APCs (0.1×10^5) and T-cell clones (0.5×10^5) were incubated with carbamazepine, carbamazepine 10,11 epoxide, 10-hydroxy carbamazepine, oxcarbazepine, and N-acetyl iminostilbene (all 10-100 μg/mL). Proliferation was measured by incorporation of [^{3}H] thymidine.

Generation of 3-dimensional energy-minimized molecular models of carbamazepine (metabolites)

Energy minimization is a computational technique based on a theoretical model using Newtonian mechanics, which locates an energy minimum (strain-free) confirmation for a molecule. Minimizations were performed by using Cerius^2 (version 4.6, Cerius^2 Modelling Environment; Molecular Simulations Inc, San Diego, Calif) software running on a Silicon Graphics O_2 workstation (http://www.sgi.com; Silicon Graphics Inc, Reading, United Kingdom). All calculations performed used the default settings in Cerius^2 software. The molecules were built and partial charges calculated by using the polymer-consistent force field.[12] The compounds were minimized in the polymer-consistent force field by using 4 minimization algorithms sequentially (Steepest Descent, Newton Raphson methods: Adopted Base Newton-Raphson, Quasi-Newton-Raphson, Truncated Newton-Raphson).[13] All of the models were aligned with reference to 1 benzene ring.

Investigation of the mechanism of carbamazepine (metabolite) presentation to T cells

The role of processing in carbamazepine (metabolite) presentation was determined by chemical fixation of APCs with glutaraldehyde (0.05%; 30 seconds) and measurement of the kinetics of T-cell receptor internalization, which is an early paramcter of antigen T-cell receptor engagement. To determine the time-course of T-cell receptor internalization, carbamazepine (metabolite)–stimulated T-cell clones were stained with a fluorescein isothiocyanate–labeled anti-CD3 mAb after 1, 4 and 16 hours and analyzed by flow cytometry. Values of 100% represent the mean CD3 fluorescence of T cells incubated with APCs in the absence of drug. Rapid T-cell receptor internalization (less than 4 hours) is indicative of processing independent drug presentation.[14]

Determination of the role of metabolism by immune cells in carbamazepine (metabolite) presentation to T cells

1-Aminobenzotriazole (1 mmol/L), a mechanism-based inhibitor of haem-containing oxygenases,[15] and N-acetyl cysteine (1 mmol/L) or glutathione (1 mmol/L), which bind irreversibly to protein-reactive carbamazepine metabolites,[16] were added to the proliferation assay. In addition, pulsing experiments that involve the incubation of carbamazepine with APCs (0.2×10^6) for 1 to 2 hours, followed by repeated washing steps to remove noncovalently bound drug before the addition of the APCs to T cells, were performed as described previously.[6]

Investigation of the kinetics of carbamazepine-specific T-cell activation

To evaluate the activation threshold for T-cell proliferation for an individual clone, a panel of T-cell clones (1×10^6) was incubated with APCs (0.2×10^6) and carbamazepine (25 μg/mL; total volume, 1 mL) for 0.1, 1, 2, 4, and 16 hours. At each time point, cells were washed repeatedly (3×4 mL), suspended in drug-free media, and incubated for a further 48 hours before determination of proliferation by incorporation of [^{3}H] thymidine.

Statistical analysis

The Mann-Whitney test was used for comparison of control and test values, accepting $P < .05$ as significant.

RESULTS

Carbamazepine and certain stable carbamazepine metabolites stimulate lymphocyte proliferation

Lymphocytes from 11 (out of 12) carbamazepine-hypersensitive patients proliferated *in vitro* with

TABLE I. Clinical details of hypersensitive patients

	Age (y)	Sex	Days to reaction	Details of reaction	Months since reaction	Rechallenge
1*	70	M	21	Widespread maculopapular eruption with tissue eosinophilia	28	No
2	66	F	42	Maculopapular rash, fever, eosinophilia, abnormal LFTs	84	No
3	33	M	21	Exfoliative dermatitis, facial swelling, fever, and eosinophilia	180	No
4	67	F	6	Widespread erythematous rash, lymphocytosis, and eosinophilia	180	No
5	31	M	28	Erythematous rash, jaundiced, hepatomegaly	160	No
6	33	M	21	Widespread severe skin rash	21	No
7	27	M	21	Widespread rash with fever, history of allergy to multiple drugs	36	No
8	40	F	21	Widespread maculopapular erythematous rash	6	No
9	55	F	21	Widespread maculopapular rash, similar reaction to lamotrigine	12	No
10	65	F	Rechallenge	Desquamating rash with fever on inadvertent rechallenge, history of rash on initial exposure	<1	Positive
11	28	F	Rechallenge	Widespread maculopapular rash with fever/eosinophilia after 1 dose, history of allergy to antibiotics	2	Positive
12	31	M	12	Widespread severe maculopapular rash with fever	229	No
13	67	F	22	Generalized maculopapular rash	23	No

M, Male; *F*, female; *LFTs*, liver function tests.
*Number assigned to patient throughout manuscript (patient 13 exposed to oxcarbazepine).

TABLE II. Carbamazepine-specific proliferation of lymphocytes from hypersensitive patients

		Drug (μg/mL)				
	cpm in control	5	10	25	50	100
1*	584	5.2	7.5	9.8	3.3	1.0
2	289	8.5	44.3	42.1	22.8	2.1
3	1003	3.1	14.3	18.7	29.8	5.1
4	1443	6.0	12.5	10.5	13.5	2.1
5	1078	2.8	8.4	6.4	4.9	0.5
6	1321	1.5	2.3	3	1.6	0.3
7	687	8.1	7.1	7.6	4.2	1.5
8	399	9.2	17.6	23.4	18.9	4.2
9	881	11.5	13.8	24.7	9.9	1.2
10	670	22.5	69.4	42.3	38.7	3.2
11	1004	16.4	28.6	23.2	6.4	1.4
12	1294	1.1	0.9	1.2	1.0	1.1
13	920	2.8	4.8	5.3	4.2	1.7
Mean		7.6	17.8	16.8	12.2	2.0
SD		6.0	18.7	13.3	11.5	1.4

Data presented as SI (coefficient of variation consistently less than 20%).
*Number assigned to patient throughout manuscript (patient 13 exposed to oxcarbazepine).

carbamazepine (Table II). Lymphocytes from 5 patients were also incubated with stable carbamazepine metabolites. Carbamazepine 10,11 epoxide, 10-hydroxy carbamazepine, and oxcarbazepine, but not 2-hydroxy carbamazepine, 3-hydroxy carbamazepine, carbamazepine 10,11 *cis*-diol, or carbamazepine 10,11 *trans*-diol (SI 0.8-1.3), resulted in significant lymphocyte proliferation compared with vehicle alone ($P < .05$; Fig 1, *A*). N-acetyl iminostilbene stimulated low levels of lymphocyte prolifcration (Fig 1, *B*; $P < .05$). A qualitatively similar pattern of proliferation was observed with lymphocytes from the oxcarbazepine-hypersensitive patient (patient 13). Lymphocytes proliferated in the presence of carbamazepine, carbamazepine 10,11 epoxide, 10-hydroxy carbamazepine, and oxcarbazepine (Fig 1, *A*), but not other carbamazepine metabolites (SI < 1.5). Lymphocytes from carbamazepine-tolerant patients (SI 1.2 ± 0.4 [carbamazepine 25 μg/mL]) and carbamazepine-naive volunteers (SI 1.1 ± 0.2 [carbamazepine 25 μg/mL]) did not proliferate with carbamazepine or carbamazepine metabolites (SI < 1.5).

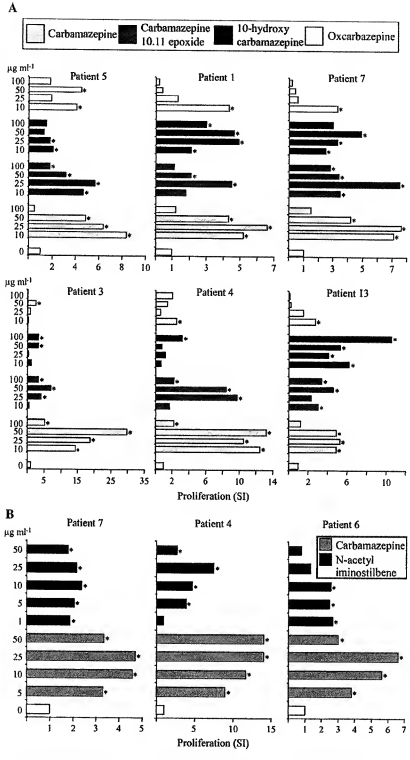

FIG 1. Proliferation of lymphocytes from hypersensitive patients stimulated with **(A)** carbamazepine, oxcarbazepine, and carbamazepine metabolites and **(B)** N-acetyl iminostilbene. Coefficient of variation was consistently less than 20%.

TABLE III. Proliferation of T-cell clones from patients hypersensitive to carbamazepine and oxcarbazepine

	Carbamazepine-hypersensitive patients						Oxcarbazepine-hypersensitive patient		
	Patient 3			Patient 7			Patient 13		
	n*	0	25 μg/mL	n*	0	25 μg/mL	n*	0	25 μg/mL
Carbamazepine	24	1755 ± 1119	6416 ± 4355	29	2350 ± 2650	9683 ± 7479	11	580 ± 449	3585 ± 3221
Carbamazepine 10,11 epoxide	18	1164 ± 1395	3647 ± 3010	7	16,239 ± 13,920	27,907 ± 21,217	6	141 ± 57	686 ± 424
10-Hydroxy carbamazepine	32	1884 ± 1174	14,383 ± 13,001	12	746 ± 228	15,986 ± 9627	13	2176 ± 2300	9664 ± 8795
Oxcarbazepine	5	1772 ± 923	4565 ± 1759	25	6874 ± 552	19,656 ± 14,206	5	1432 ± 943	4191 ± 2950

Data presented as mean cpm ± SD.
*Number of clones that proliferate with a given compound.

Generation and characterization of carbamazepine and carbamazepine metabolite–specific T-cell clones

Greater than 100 carbamazepine-specific T-cell clones were generated from 6 hypersensitive patients (0, 4981.5 ± 5846.4 cpm; carbamazepine 25 μg/mL, 17721.4 ± 15964.9 cpm [patients 1, 2, 3, 4, 6 and 7]). Although the vast majority of these T-cell clones were $CD4^+$, 11 clones stained double-positive (n = 4) or were $CD8^+$ (n = 7). T-cell clones expressed a single αβ T-cell receptor; however, the αβ T-cell receptor repertoire was heterogeneous. All of the clones responded to carbamazepine in a dose-dependent manner.

In subsequent experiments, lymphocytes from 2 carbamazepine (patients 3 and 7) and 1 oxcarbazepine-hypersensitive patient (patient 13) were stimulated with carbamazepine, carbamazepine metabolites, and oxcarbazepine and cloned by serial dilution. 53, 25, 44, and 30 (carbamazepine-hypersensitive patients) and 11, 6, 13, and 5 (oxcarbazepine-hypersensitive patient) clones were found to proliferate with carbamazepine, carbamazepine 10,11 epoxide, 10-hydroxy carbamazepine, and oxcarbazepine, respectively (Table III).

Cross-reactivity patterns of carbamazepine and carbamazepine metabolite–specific T-cell clones

After clonal expansion, 29 well growing clones from patients 3, 7, and 13 were chosen to perform detailed structure activity studies. Four patterns of drug recognition were observed. First, 13 T-cell clones (1 carbamazepine, 5 carbamazepine 10,11 epoxide, and 7 oxcarbazepine-specific) proliferated only in the presence of the compound that the lymphocytes were initially stimulated with. Second, 5 T-cell clones (3 carbamazepine 10,11 epoxide and 2 oxcarbazepine-specific) proliferated in the presence of carbamazepine, carbamazepine 10,11 epoxide, 10-hydroxy carbamazepine, and oxcarbazepine. Third, 6 T-cell clones proliferated with carbamazepine and 10,11 epoxide (4 carbamazepine and 2 carbamazepine 10,11 epoxide–specific), but not 10-hydroxy carbamazepine or oxcarbazepine. Finally, 5 T-cell clones proliferated with 10-hydroxy carbamazepine and oxcarbazepine (4 10-hydroxy carbamazepine and 1 oxcarbazepine), but not carbamazepine or carbamazepine 10,11 epoxide. Results from 8 T-cell clones depicting each pathway of drug recognition are shown in Fig 2, *A*.

Data presented in Fig 2, *B*, show the preferred 3-dimensional spatial arrangement of carbamazepine and carbamazepine metabolites. The structures of carbamazepine and carbamazepine 10,11 epoxide, with the exception of the epoxide moiety, were almost identical. Similarly, 10-hydroxy carbamazepine and oxcarbazepine are structurally similar with the exception of the ketone and alcohol functionalities. However, the 3-dimensional spatial arrangement of atoms within these 2 groups of compounds differs significantly (ie, the tricyclic regions of 10-hydroxycarbamazepine and oxcarbazepine are more highly twisted).

Carbamazepine and carbamazepine metabolites are presented to T-cells in the absence of antigen processing and immune cell metabolism

Carbamazepine, carbamazepine metabolites, and oxcarbazepine stimulated T-cell proliferation when presented in the context of fixed APCs (Fig 3, *A*). Similarly, a rapid downregulation (within 1 hour) of T-cell receptor expression was observed after drug (metabolite) stimulation (Fig 3, *B*). Carbamazepine (metabolite)–pulsed APCs did not stimulate carbamazepine, carbamazepine 10,11 epoxide, 10-hydroxy carbamazepine, or oxcarbazepine-specific T-cell clones (Fig 3, *A*). Furthermore, addition of glutathione, 1-aminobenzotriazole, or N-acetyl cysteine did not inhibit the T-cell response.

Rapid stimulation of carbamazepine-specific T-cell clones

To define the activation threshold required for effective T-cell receptor stimulation, APCs and T cells were pulsed together with carbamazepine for 0.1 to 16 hours. Here, carbamazepine forms a complex with MHC and the T-cell receptor before washing. These experiments show that the minimum drug exposure to APCs and T cells for effective T-cell proliferation is 0.1 and 4 hours (Fig 3, *C*).

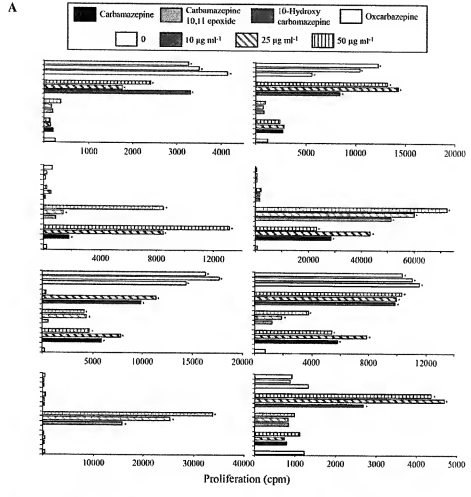

FIG 2. A, Proliferation of representative T-cell clones from patients 3, 7, and 13 with carbamazepine, oxcarbazepine, and carbamazepine metabolites. Coefficient of variation was consistently less than 20%. B, Generation of energy minimized molecular models of carbamazepine, oxcarbazepine, and carbamazepine metabolites.

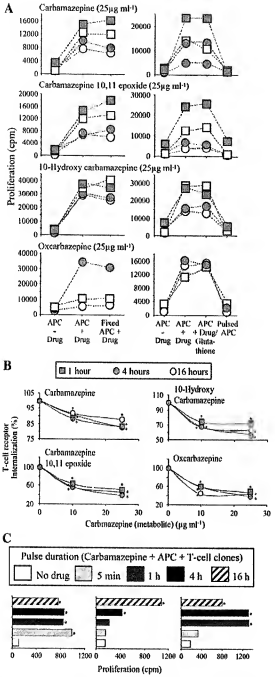

FIG 3. Carbamazepine and carbamazepine metabolites are presented to T-cell clones rapidly in the absence of antigen processing and metabolism. **A**, T-cell clones were incubated with irradiated or fixed APCs and carbamazepine (metabolites; *column 1*). Glutathione was added to certain incubations *(column 2)*. Pulsing APCs alone with carbamazepine (drug-treated cells with drug washed out) did not stimulate proliferation *(column 2)*. *Dotted lines* link data from individual clones. **B**, Kinetics of T-cell receptor internalization after stimulation with carbamazepine (metabolites). **C**, Determination of the minimum length of carbamazepine exposure required for stimulation of T-cell clones. Carbamazepine was pulsed with APCs and T cells together for various periods before washing and determination of proliferation. Coefficient of variation was consistently less than 20%.

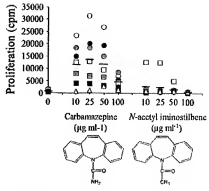

FIG 4. Proliferation of T-cell clones with carbamazepine and N-acetyl iminostilbene. Each shape depicts data from an individual T-cell clone. Coefficient of variation was consistently less than 20%.

Stimulation of carbamazepine-specific T-cell clones with N-acetyl iminostilbene

A panel of carbamazepine-specific T-cell clones from patients 2, 3, 4, and 13 was stimulated with carbamazepine and N-acetyl iminostilbene to determine the importance of the amine functionality in the binding of carbamazepine to MHC and/or the T-cell receptor. N-acetyl iminostilbene is a deamino-methyl substituted derivative of carbamazepine. Six out of 7 T-cell clones proliferated in the presence of carbamazepine alone. N-acetyl iminostilbene-specific proliferation was detected with only 1 clone (Fig 4).

DISCUSSION

Carbamazepine hypersensitivity syndrome is a rare but severe adverse drug reaction. The detection of drug-specific lymphocytes from hypersensitive patients suggest that the reaction is immune-mediated.[17-20] Herein, we address the issue of T-cell responses to stable carbamazepine metabolites and evaluate whether clinical cross-reactivity between drugs may relate to the generation of metabolite-specific T-cells. The principal pathways of carbamazepine metabolism in human beings involve formation of the chemically stable 10,11 epoxide metabolite and subsequent hydrolysis products. Minor pathways include hydroxylation of the aromatic rings.[21-23] Several studies have shown that carbamazepine is also metabolized to a protein reactive metabolite in human liver microsomes[16,24,25]; however, the structure of the reactive species remains somewhat controversial. Lymphocytes from 11 out of 12 hypersensitive patients were found to proliferate with therapeutic concentrations of carbamazepine (5-10 μg/mL).[26] The proliferative response with certain patients cells was stronger (SI 20-70; Table II) than that published previously, which emphasizes the importance of using well defined cell culture methodologies and experienced laboratory scientists. The strength of the response was not associated with specific clinical symptoms or the time since the reaction occurred. Carbamazepine 10,11

epoxide and 10-hydroxy carbamazepine, but not other carbamazepine metabolites, also stimulated lymphocyte proliferation. These data reveal that mono oxygenation in the same position can be accommodated by the MHC T-cell receptor binding site; however, the addition of 2 hydroxyl groups inhibits binding to either MHC or T-cell receptor.

To explore whether the observed lymphocyte proliferation with carbamazepine 10,11 epoxide or 10-hydroxy carbamazepine represents simple cross reactivity with the parent drug or the presence of carbamazepine metabolite-specific T cells in the peripheral circulation of hypersensitive patients, carbamazepine and carbamazepine metabolite-specific T cells were cloned and characterized. Although approximately 30% of carbamazepine metabolite-specific T-cell clones proliferated in the presence of the parent drug, the majority of clones was highly specific, and proliferation was observed only with carbamazepine metabolites. These data show that a variety of T cells exist in the peripheral circulation of hypersensitive patients, some of which stimulate selectively stable carbamazepine metabolites. In line with previous studies,[6-8] carbamazepine and carbamazepine metabolites were presented to T cells directly in the absence of immune cell metabolic activation and/or processing. This form of drug antigen presentation has some similarities to superantigen stimulation, a topic that has been reviewed in detail elsewhere.[27] The threshold drug incubation period for a detectable T-cell response for a given clone varied between 0.1 and 4 hours, which may represent the time taken to ligate a threshold number of T-cell receptors.[28]

Interestingly, when 29 T-cell clones were stimulated with carbamazepine, carbamazepine metabolites, and oxcarbazepine (a keto derivative of carbamazepine), several clones were stimulated by only 1 compound, whereas further clones could accommodate carbamazepine, oxcarbazepine, and carbamazepine metabolites. Certain clones also displayed an unexpected cross-reactivity profile: proliferation was seen with carbamazepine and carbamazepine 10,11 epoxide or 10-hydroxy carbamazepine and oxcarbazepine (Fig 3). Each pattern of drug recognition was detected by using clones from patients with Stevens-Johnson syndrome (patient 3) and generalized hypersensitivity syndrome (patients 7 and 13). Generation of *in silico* energy minimized molecular models revealed that carbamazepine and carbamazepine 10,11 epoxide and 10-hydroxy carbamazepine and oxcarbazepine can adapt structures with a similar spatial arrangement of atoms, thereby providing 1 possible explanation for the recognition of carbamazepine and carbamazepine metabolites by T cells. Alternatively, it is possible that drug (metabolites) with different functional groups actually binds to different HLA molecules or to different peptides in the MHC groove. This possibility requires further investigation.

The importance of the terminal amine functionality in the interaction of carbamazepine with MHC or specific T-cell receptors was determined by incubating N-acetyl iminostilbene with lymphocytes and T-cell clones. A stimulation index of greater than 2.5 was observed when lymphocytes from only 1 out of 3 hypersensitive patients were incubated with N-acetyl iminostilbene. Moreover, only 1 out of 7 carbamazepine-specific T-cell clones proliferated in the presence of N-acetyl iminostilbene. These data suggest that the amine group is important for the binding of carbamazepine to MHC or specific T-cell receptors, presumably through the formation of ionic or hydrogen bonding interactions.

After withdrawal of carbamazepine, hypersensitive patients often require alternative anticonvulsant medication. This is often problematic because patients hypersensitive to 1 anticonvulsant may develop a hypersensitivity reaction to the alternative medication.[29] Zakrzewska and Ivanyi[17] have reported that lymphocytes from carbamazepine-hypersensitive patients are not stimulated by oxcarbazepine, and therefore suggested that oxcarbazepine may be suitable as a safe alternative drug treatment. However, clinical data show that approximately 25% of carbamazepine-hypersensitive patients cross-react with oxcarbazepine *in vivo*.[30] Importantly, oxcarbazepine is a pro-drug that is rapidly converted to 10-hydroxy carbamazepine *in vivo*.[31] Our data using cells from carbamazepine-hypersensitive and oxcarbazepine-hypersensitive patients show that proliferation of lymphocytes can be detected in the presence of carbamazepine, oxcarbazepine, and 10-hydroxy carbamazepine. Thus, oxcarbazepine administration to carbamazepine-hypersensitive patients may lead to the reactivation of preexisting memory T-cells and the development of a hypersensitivity reaction.

In conclusion, these studies show that patients have a range of T cells, some of which are specific for stable carbamazepine metabolites. These results are compatible with the pharmacologic interactions concept for activation of drug-specific T cells proposed by Pichler.[32]

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REFERENCES

1. Leyva L, Torres MJ, Posadas S, Blanca M, Besso G, O'Valle F, et al. Anticonvulsant-induced toxic epidermal necrolysis: monitoring the immunologic response. J Allergy Clin Immunol 2000;105:157-65.
2. Farrell J, Gibney CA, Pirmohamed M, Park BK, Naisbitt DJ. Characterization of drug-specific CD8(+) T-cells expressing the skin homing receptor CCR10 in lamotrigine hypersensitivity. Br J Clin Pharmacol 2004;57:690.
3. Naisbitt DJ, Britschgi M, Wong G, Farrell J, Depta JP, Chadwick DW, et al. Hypersensitivity reactions to carbamazepine: characterization of the specificity, phenotype, and cytokine profile of drug-specific T cell clones. Mol Pharmacol 2003;63:732-41.
4. Landsteiner K, Jacobs J. Studies on the sensitization of animals with simple chemical compounds. J Exp Med 1935;61:643-56.
5. Naisbitt DJ, Farrell J, Gordon SF, Maggs JL, Burkhart C, Pichler WJ, et al. Covalent binding of the nitroso metabolite of sulfamethoxazole leads to toxicity and major histocompatibility complex-restricted antigen presentation. Mol Pharmacol 2002;62:628-37.
6. Schnyder B, Burkhart C, Schnyder-Frutig K, Von Greyerz S, Naisbitt DJ, Pirmohamed M, et al. Recognition of sulfamethoxazole and its

reactive metabolites by drug-specific CD4+ T cells from allergic individuals. J Immunol 2000;164:6647-54.
7. Burkhart C, Von Greyerz S, Depta JP, Naisbitt DJ, Britschgi M, Park BK, et al. Influence of reduced glutathione on the proliferative response of sulfamethoxazole-specific and sulfamethoxazole-metabolite-specific human CD4+ T-cells. Br J Pharmacol 2001;132:623-30.
8. Naisbitt DJ, Farrell J, Wong G, Depta JP, Dodd CC, Hopkins JE, et al. Characterization of drug-specific T cells in lamotrigine hypersensitivity. J Allergy Clin Immunol 2003;111:1393-403.
9. Burkhart C, Britschgi M, Strasser I, Depta JP, von Greyerz S, Barnaba V, et al. Non-covalent presentation of sulfamethoxazole to human CD4+ T cells is independent of distinct human leucocyte antigen-bound peptides. Clin Exp Allergy 2002;32:1635-43.
10. Pichler WJ, Tilch J. The lymphocyte transformation test in the diagnosis of drug hypersensitivity. Allergy 2004;59:809-20.
11. Schnyder B, Mauri-Hellweg D, Zanni M, Bettens F, Pichler WJ. Direct, MHC-dependent presentation of the drug sulfamethoxazole to human alphabeta T cell clones. J Clin Invest 1997;100:136-41.
12. Maple JR, Hwang MJ, Stockfisch TP, Dinur U, Waldman M, Ewig CS, et al. Derivation of class-II force fields, 1: methodology and quantum force field for the alkyl functional group and alkane molecules. J Comput Chem 1994;15:162-82.
13. Levitt M, Lifson S. Refinement of protein conformations using a macromolecular energy minimization procedure. J Mol Biol 1969;46:269-79.
14. Zanni MP, von Greyerz S, Schnyder B, Brander KA, Frutig K, Hari Y, et al. HLA-restricted, processing- and metabolism-independent pathway of drug recognition by human alpha beta T lymphocytes. J Clin Invest 1998;102:1591-8.
15. Ortiz De Montellano PR, Mathews JM, Langry KC. Autocatalytic inactivation of cytochrome P-450 and chloroperoxidase by 1-aminobenzotriazole and other aryne precursors. Tetrahedron 1984;40:511-9.
16. Madden S, Maggs JL, Park BK. Bioactivation of carbamazepine in the rat in-vivo: evidence for the formation of reactive arene oxide(s). Drug Metab Dispos 1996;24:469-79.
17. Zakrzewska JM, Ivanyi L. In vitro lymphocyte proliferation by carbamazepine, carbamazepine-10,11-epoxide, and oxcarbazepine in the diagnosis of drug-induced hypersensitivity. J Allergy Clin Immunol 1988;82:110-5.
18. Mauri-Hellweg D, Bettens F, Mauri D, Brander C, Hunziker T, Pichler WJ. Activation of drug-specific CD4+ and CD8+ T cells in individuals allergic to sulfonamides, phenytoin, and carbamazepine. J Immunol 1995;155:462-72.
19. Brown KL, Henderson DC, Nadel S, Tanveer A, Booy R. Carbamazepine hypersensitivity and the use of lymphocyte proliferation responses. Dev Med Child Neurol 1999;41:267-9.
20. Hari Y, Frutig-Schnyder K, Harni M, Yawalkar N, Zanni MP, Schnyder B, et al. T cell involvement in cutaneous drug eruptions. Clin Exp Allergy 2001;31:1398-408.
21. Pearce RE, Vakkalagadda GR, Leeder JS. Pathways of carbamazepine bioactivation in vitro I: characterization of human cytochromes P450 responsible for the formation of 2- and 3-hydroxylated metabolites. Drug Metab Dispos 2002;30:1170-9.
22. Maggs JL, Pirmohamed M, Kitteringham NR, Park BK. Characterization of the metabolites of carbamazepine in patient urine by liquid chromatography/mass spectrometry. Drug Metab Dispos 1997;25:875-80.
23. Pienimaki P, Hartikainen AL, Arvela P, Partanen T, Herva R, Pelkonen O, et al. Carbamazepine and its metabolites in human perfused placenta and in maternal and cord blood. Epilepsia 1995;36:241-8.
24. First SM, Uetrecht JP. The effect of carbamazepine and its reactive metabolite, 9-acridine carboxaldehyde, on immune cell function in vitro. Int J Immunopharmacol 1995;17:445-52.
25. Ju C, Uetrecht JP. Detection of 2-hydroxyiminostilbene in the urine of patients taking carbamazepine and its oxidation to a reactive iminoquinone intermediate. J Pharmacol Exp Ther 1999;288:51-6.
26. Tomson T, Tybring G, Bertilsson L, Ekbom K, Rane A. Carbamazepine therapy in trigeminal neuralgia: clinical effects in relation to plasma concentration. Arch Neurol 1980;37:699-703.
27. Pichler WJ. Pharmacological interaction of drugs with antigen-specific immune receptors: the p-i concept. Curr Opin Allergy Clin Immunol 2002;2:301-5.
28. Depta JP, Altznauer F, Gamerdinger K, Burkhart C, Weltzien HU, Pichler WJ. Drug interaction with T-cell receptors: T-cell receptor density determines degree of cross-reactivity. J Allergy Clin Immunol 2004;113:519-27.
29. Shear NH, Spielberg SP, Cannon M, Miller M. Anticonvulsant hypersensitivity syndrome: in vitro risk assessment. J Clin Invest 1988;82:1826-32.
30. Jenson NO, Damm M, Jakobson K. Oxcarbazepine in patients hypersensitive to carbamazepine. Irish J Med Sci 1986;155:297.
31. Dickinson RG, Hooper WD, Dunstan PR, Eadie MJ. First dose and steady-state pharmacokinetics of oxcarbazepine and its 10-hydroxy metabolite. Eur J Clin Pharmacol 1989;37:69-74.
32. Pichler WJ. Delayed drug hypersensitivity reactions. Ann Intern Med 2003;139:683-93.